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APPLICATION AND POTENTIAL OF COMBINATORIAL TECHNOLOGY TO LIPID /OIL RESEARCH AND DEVELOPMENT

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Research and development for the optimization of processing schemes is costly and time-consuming, particularly when many parameters must be evaluated which have an impact on the final desired result. To overcome the above problems, several approaches can be used to save on the time and labor involved in process optimization, including theoretical prediction, statistical optimization, and combinatorial experimentation. The first two of these options have been utilized for some time now by researchers, however the use of a combinatorial methodology or approach is somewhat new, and the subject of this article.

The combinatorial approach has not always been accepted by researchers since it is perceived by some as a "brute" force method in which all the relevant combinations of experimental parameters are evaluated without resorting to logic and scientific intuition. Whereas combining the above approaches has been advocated and demonstrated in the scientific literature [1], the rapid development in terms of the speed and automation of analytical techniques, coupled with developments in miniaturization and fluidics have so increased experimental throughput, that the combinatorial approach should be of value to researchers in the field of oil and lipid technology. In this brief article, we shall illustrate how the complexity of processing and analyzing lipid-based materials can be simplified by the use of the combinatorial approach, particularly when many parameters must be evaluated and screened by the researcher.

The mention of the word "combinatorial" usually conjures up the image to many of the high throughput screening of synthetic compound libraries or natural products in the pharmaceutical industry [2]. Indeed, this is probably the best known as well as time-honored application of the combinatorial method. However more recently, a number of

other applications for the combinatorial assessment of experimental parameters have appeared in the literature [3], such as:

screening of catalysts and catalytic activity

evaluation of sorbents for chromatographic selectivity

optimization of chiral selectors

assessment of electrode activity and reactions

composition of materials (e.g., solid state or semiconductor properties)

analytical method optimization

improving the efficiency and optimization of industrial processes

It is impossible here to discuss all of the above applications in this brief review, however we shall focus here on application areas and approaches which have relevance to the oil/lipid technologist, using as examples, the author's and his colleagues research in supercritical fluid extraction, fractionation, and reaction technology as they relate to lipid materials. Among these applications in which a combinatorial approach has been used are the extraction of vegetable or essential oils, the fractionation and enrichment of

high value lipid moieties for nutraceuticals and functional food application, optimization of reaction conditions or selection of catalysts, and in analytical method development. Specific methodology and approach can be reviewed in the author's list of publications at the following web site: www.scrub.lanl.gov

Types of Experimental Combinatorial Processing or Evaluation

Experimental combinatorial approaches and techniques can generally be divided into the following categories:

batch or multi-well reactors, etc.

sequential

parallel

pooled approach

We shall discuss and illustrate the first three options; the last method is mostly employed in the pharmaceutical industry for the synthesis of compounds on solid state supports [1].

The batch or multi-well approach makes use of wells or discrete reactor volumes in which the composition of several extractions/reactions can be studied under the same conditions (i.e., temperature or pressure) while one or more of the experimental variables is changed in a consistent manner for all of the samples and systems under study. Experimental equipment for such an approach can be assembled in the laboratory or purchased from commercial vendors (see the partial listing at the end of this article). Sequential methods make use of the rapid sequencing of experiments, such as

extractions or reactions, in a controlled fashion, individually. This will be illustrated below for the supercritical fluid fractionation (SFF) of phospholipids from crude lecithin or vegetable oil with the aid of microprocessor-controlled instrumentation.

Parallel assessment and optimization of processing or analysis conditions is most often done on samples having different compositions (or reactants), although in principle these can also be varied along with the external experimental conditions. For example in Figure 1, a commercial parallel reactor available from Argonaut Technologies is shown which allows up to eight independent reactions to be conducted under different specific conditions simultaneously at temperatures ranging from ambient to 200°C and a pressure range extended up to 500 psig (34 bar). This approach can also be achieved by using a series of identical individual reactor modules "piggy-backed" together as shown in Figure 2a. Here the Parr Model 5000 multiple reactor system can be operated at pressures up to 3000 psig (204 bar) along with individual control of the reactor temperature to 300°C for six 75 mL stirred reactors . The entire system can be computer controlled along with data logging of such variables as pressure. Custom designed systems such as the multi-vessel unit shown in Figure 2b are also available. Other commercial options exist and are noted at the conclusion of this article. A supercritical fluid extraction (SFE) system analogue of the above reaction systems has been designed by King and Hopper [5]. Utilizing this system it is possible to conduct eight extractions or reactions simultaneously at different pressures and flow rates at a common temperature. Details on this parallel SFE/reaction system can be found in the literature [6] where it has primarily been used to extract in parallel, multiple samples for total fat or pesticide residue analysis.

Examples of Combinatorial Assessment Related to Critical Fluid Technology

As an example illustrating how combinatorial methodology can be applied to advantage in oil/lipid research and process development, we have chosen to show its application to our research in supercritical fluid technology. As indicated in Table I, both reactions and various fractionations or extractions can be conducted in compressed fluids, but there are a number of parameters which must be investigated to optimize a specific process. For example, when conducting a reaction in supercritical fluid media such as carbon dioxide ($SC\text{-}CO_2$) it is necessary to see if the reaction is even feasible, and subsequently to evaluate its kinetics. Likewise the screening of catalysts and their impact on the above two aspects of reactions can be done rapidly using combinatorial techniques [7]. Specific examples that have utilized a sequential approach include testing the feasibility of methylating steryl esters (SE) and phospholipids (PL), as well as the evaluation of an enzymatic activity for lipase catalysts (see below).

SFE can be optimized by rapidly assessing the effect of extraction fluid pressure and temperature (and therefore fluid density) on the yield of an extract or its composition. Similarly, the effect of time and preparation of substrate (moisture, aging, comminution) on extract yield and the rate of extraction can be assessed. In our laboratory, the use of sequential combinatorial techniques have been applied for the SFE of cedarwood oil, PL extraction from soybean meal, and to corn bran or fiber extractions. For example, in the SFE of cedarwood oil, the effect of: six different pressure levels from 193 to 690 bar, three different temperatures, the extraction time, as well as substrate type or parameters, were all evaluated using a sequential combinatorial method. From these experiments the

most optimal extraction conditions were rapidly established saving consider time and labor.

The use of supercritical fluids for the fractionation and enrichment of high value lipid moieties can also be studied and optimized using combinatorial technology. Some examples include the fractionation of rice bran deodorizer distillate and/or phospholipids derived from lecithin or soybean oil. In the latter case, a multi-unit process employing an initial SFE step using neat SC-CO_2 followed by SC-CO_2 extraction utilizing ethanol as co-solvent, and than followed by preparative supercritical fluid chromatography (SFC), is required to obtain significantly enriched PL fractions [8]. The SFC step requires optimization of a number of experimental parameters , including pressure, temperature, type of sorbent , eluent type and its composition and level. These were rapidly assessed sequentially using a microprocessor-controlled Isco Model 3560 extractor (Isco, Inc., Lincoln, NE). This SFE unit allowed fractions to be collected at various stages of eluent programming over constant time periods followed by HPLC analysis.

Sorbents were quickly screened by placing different types in the extraction cells in the Isco unit. Sorbents evaluated included silica gel, several different types of alumina, diatomaceous earths, and functionalized silica packings. Depending on the desired result, either silica gel and neutral or basic alumina were found to be optimal. Additional runs using various combinations of water with ethanol at various percentages in SC-CO_2 as the chromatographic mobile phase allowed the fine tuning of SFF process. These SFF experiments proceeded scaled up runs of SFC process step, resulting in a fractionation that produced 55-75% pure fractions of specific PLs, thereby saving

considerable time and effort in process development. Extensions of this approach can also be used for the SFF of phytosterols and phytosterol esters.

The evaluation of an appropriate catalyst and conditions for its use in SC-CO_2 can also be assessed in a combinatorial manner using both automated sequential and parallel analyzers. Enzymatic synthesis in supercritical fluids attracted considerable interest due to the "green" nature of the process [9], particularly for the conversion and processing of lipid-related materials. We have studied previously the transesterification of various lipid species and shown that with the proper selection of reaction conditions and catalyst, that rapid and complete transesterifications can be achieved. These studies have been have been performed in a combinatorial fashion using a sequential analyzer similar to the Isco system described previously. In this case, the candidate supported lipases were placed in the extraction cell of the analyzer and methanol added as a cosolvent (reactant) to the flowing SC-CO_2 phase. The lipid species which will undergo transesterification to form methyl esters are placed ahead of the supported catalyst in the extraction (reaction) cell, thereby permitting solvation into the SC-CO_2/methanol mixtures, converting the SFE unit to a flow reactor module. By rapidly testing various combinations of enzymes (lipases) with specific target lipid compounds using the above technique , the activity of various lipases in the presence of SC-CO_2 can be assessed .

Table II shows the results achieved for the methylation of shortening), cholesteryl stearate , and phosphatidylcholine. As seen in Table II, a large number of lipases exhibit negligible activity in SC-CO_2, however several lipases produced high conversion of the chosen lipid substrates to their methyl esters. These results indicate that methylation in general could be carried out successfully by using Novozyme 435 and Chirazyme L-1;

while Lipase G and Lipozyme IM will also yield high conversions but are more substrate specific. It should be noted that the above transesterifications were run at 170 bar and 50°C; slightly different conditions may change the activity patterns of the various lipases tested. The author has reviewed both the process and analytical possibilities afforded by the reaction, which include the hydrolysis of various lipid materials, analysis of fat in foodstuffs, and coupling the transesterification reaction with a hydrogenation reaction to produce fatty alcohol mixtures [10].

Conclusions and Future Prospectus

In this brief review we have attempted to document the possibilities that combinatorial methodologies hold for lipid technology. Citing research results from our supercritical fluids research, we have shown how the combinatorial approach can be used to advantage in optimizing SFEs, fractionation schemes in supercritical fluid media, and reactions on lipid substrates. Extensions of the catalyst screening protocol exist, including using other analytical options (e.g., X-ray and infrared analyzers) to rapidly evaluate the activity of catalysts, such as inorganic-based hydrogenation catalysts.

Sequential-based pressurized liquid analyzers are also now available which can be utilized for combinatorial-based studies. These are but another analytical option which facilitates speeding up process development. A partial list of vendors and related useful website has been provided after the references. A useful website for those seeking more information on combinatorial technology is the NIST Combinatorial Methods Center site (http://polymers.msel.nist.gov/combi)

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Some Vendors Offering Combinatorial-Related Equipment

Advanced ChemTech (Louisville, KY), www.advancedchemtech.com – Reactors, etc.

Argonaut Technologies (Foster City, CA), www.argonaut.com – Multiple Reactors, ec

Baskerville (Manchester, UK), www.baskervilleautoclaves.co.uk – Multiple Reactors

Bruker AXS (Madison, WI), www.bruker-axs.com – X-Ray Evaluation of Catalysts

Chemspeed (East Windsor, NJ), www.chemspeed.com – Synthesis Stations

Chemical Diversity Labs (San Diego, CA), www.combisyn.com – Modular Reactors

HEL (Herts, England), www.helgroup.co.uk – Multiple Reactors

Isco, Inc. (Lincoln, NE), www.isco.com - Parallel Purification Equipment

Mettler-Toledo Bohdan (Vernon Hills, IL), – Reactors, Chromatography

Parr (Moline, IL), www.parrinst.com – Multiple Reactors

Radley's (Essex, United Kingdom), www.radleys.com – Multiple Reactor Systems

SEPIAtec (Kingwood, TX), www.sepiatec.com – Parallel Chromatography

Torial Technologies (Des Plaines, IL), www.torial.com – Catalysts Development

A very informative website that expands upon this list of vendors, including related information is www.combichemlab.com

Table I. Examples of Combinatorial Assessment Related to Critical Fluid Technology

Process	Example
Reaction	Feasibility and Kinetics
Reaction	Evaluation of Catalytic Activity
Fractionation	Sorbent Evaluation for Chromatography
Fractionation	Influence of Eluent Strength
Extraction	Effect of Fluid Density on Extract Yield and Composition
Extraction	Effect of Time of Extraction on Yield of Extract
Extraction	Preparation of Substrate (Moisture, Aging, Comminution)

Table II Lipase-Catalyzed Methanolysis for SFE/SFR Conversion of Lipids (%)

Lipase	Shortening	$C_{18}CE^{*}$	PC^{**}
Lipase PS30[a]	2	10	1
Lipase L[a]	4	1	N.R.
Lipase Ay[a]	5	1	N.R.
Lipase MAP10[a]	56	31	22
Lipase G[a]	90	100	48
Pseudomonas cepacia Lipase[b]	81	45	80
Novozyme 435[c]	100	98	99
Lipase from *C. Antarctica A.*[c]	1	N.R	N.R.
Chirazyme L-1[c]	100	98	90
Chirazyme E-1[c]	6	2	1
Lipozyme Im[c,d]	99	96	60

$C_{18}CE$ = cholesteryl stearate

PC = Phosphatidylchloine

[a]Immobilized on Accurel.

[b](Sol-gel) Reaction products included 15% monoglycerides and 19% diglycerides.

[c]Carrier-fixed (not specified by manufacturer).

[d]Reaction products included 16% monoglycerides

Figure Legend

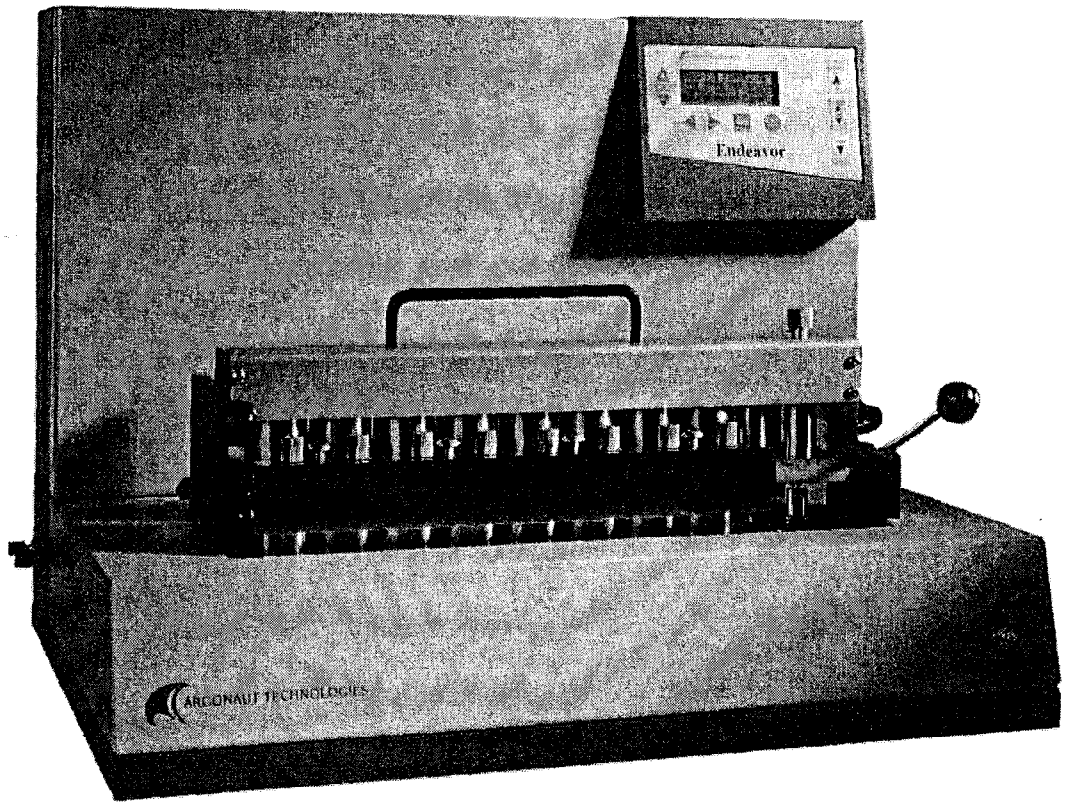
Figure 1. An Endeavor Parallel Pressure Reactor (Argonaut Technologies)

Figure 2a A Parr Instruments Series 5000 Multiple Reactor System (MRS)

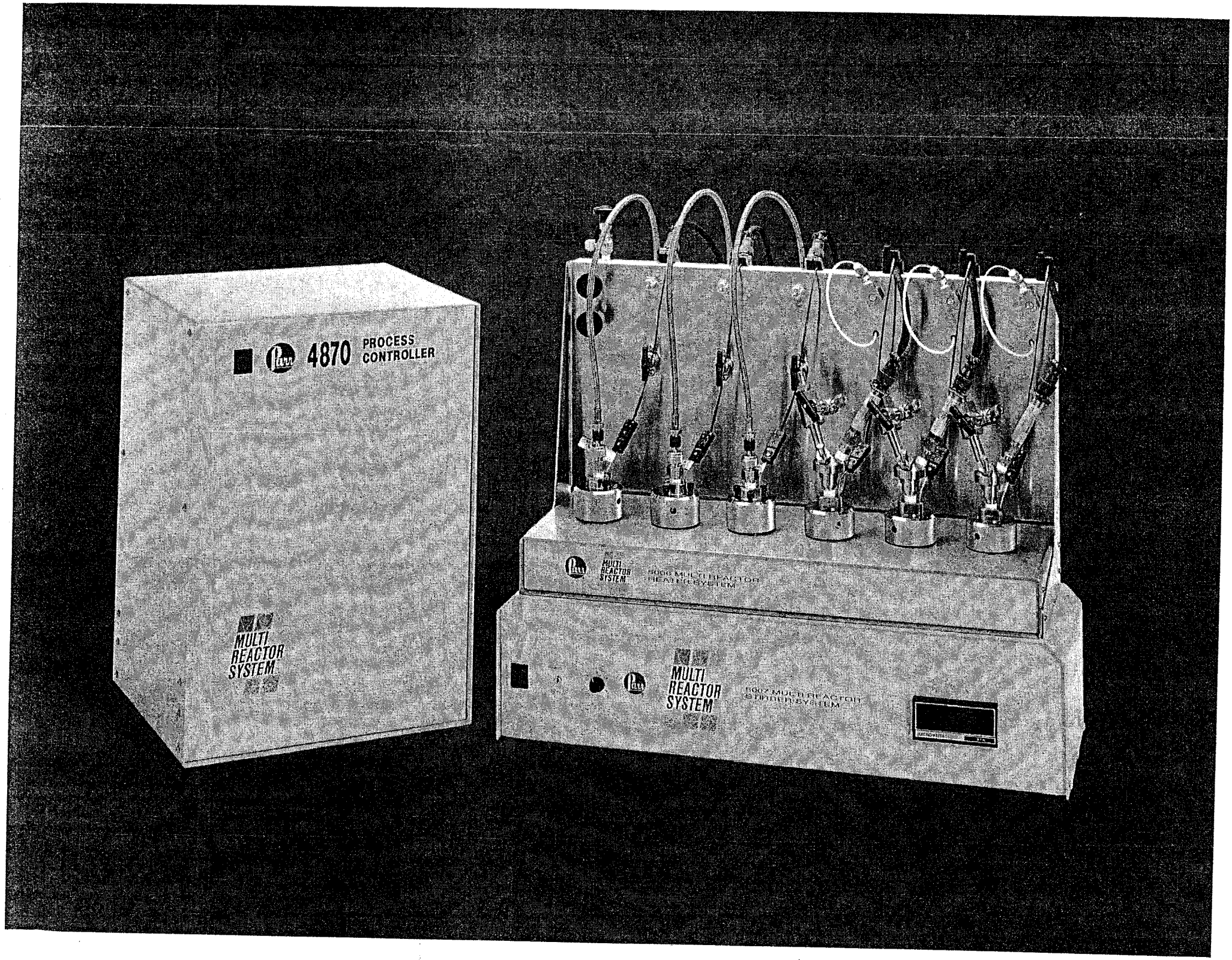
Figure 2b Custom Built Sixteen Vessel Reactor System (Courtesy Parr Instruments)

Endeavor- Parallel Pressure Reactor

- 8 independent reactors
- RT to 200° C
- 0-500 psig
- Gas uptake measurement



ARGONAUT TECHNOLOGIES


4870 PROCESS CONTROLLER
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